Saskatchewan

A D F

Agriculture Development Fund

FINAL REPORT

VALUE ADDED PROCESSING AND COMPONENTS IN DEVELOPING BLUE-AND PURPLE-PIGMENTED WHEATS

19970261

Funded by
The Agriculture Development Fund
June 1999

Prepared by: University of Saskatchewan

SAF
Saskatchewan Agriculture & Food
Adding value to agriculture...through research and development

100-05076/

ADF PROJECT 97000261 - 94BR

"VALUE ADDED PROCESSING AND COMPONENTS IN DEVELOPING BLUE- AND PURPLE-PIGMENTED WHEATS"

FINAL REPORT

AGRICULTURE DEVELOPMENT FUND
RECEIVED
JUN 11 1999
REGINA, SASK.
S4S 0J1
PROVINCE OF SASKATCHEWAN

Principal Investigator: Pierre Hucl
Co-investigator: Elsayed Abdelaal

Crop Development Centre
Department of Plant Sciences
University of Saskatchewan

EXECUTIVE SUMMARY

ABSTRACT

The composition of blue aleurone wheat anthocyanin pigments was found to be different from that of purple pericarp wheat. Four major anthocyanins were separated from blue wheat based on a three year crop analysis, compared to five anthocyanins in purple wheat. Cyanidin 3-glucoside was found to be the major anthocyanin in purple wheat, while it was the second major anthocyanin in blue wheat. The major anthocyanin in blue wheat, which constituted up to 39% of total anthocynins, needs to be identified. Modification of pH in whole blue wheat kernels and addition of sulfur dioxide would assist in stabilizing anthocyanin pigments during processing. Three naturally-colored food products were produced based on blue wheat whole meals using a drum drying process.

SUMMARY

Blue wheat, a potential naturally-colored food ingredient for the cereal industry, was investigated during the short term of this project. Processing potential and identification of individual anthocyanins and their stability were studied. Purendo 38 blue wheat samples from three crop years were analyzed for their content of individual anthocyanins by HPLC technique and compared with Konini purple wheat. Blue wheat differed in its anthocyanin profile from purple wheat. Four major anthocyanins were found in blue wheat, in addition to trace concentrations of other anthocyanins. Cyanidin 3-glucoside was found to be the second major anthocyanin in blue wheat constituting 25 to 28% of total anthocynins. Peonidin 3-glucoside was found in trace amounts in blue wheat. On the other hand, purple wheat contained five major anthocyanins with cyanidin 3-glucoside being the major member constituting up to 35% of total anthocyanins. The major anthocyanin in blue wheat, which comprised up to 39% of total anthocynins, needs to be identified.

Blue wheat anthocyanins were thermally most stable at pH 1, while their degradation was slightly lower at pH 3 as compared to pH 5. As the temperature increased from 65 to 95 °C, degradation of blue wheat anthocyanins was increased as indicated by the reduction in absorbance readings. On the other hand, addition of sulfur dioxide during heating of blue wheat had a stabilizing effect on anthocyanin pigments as compared to the control sample or heating without sulfur dioxide. The optimal SO_2 concentrations were 500-1000 ppm for whole meal and 1000-3000 ppm for isolated anthocyanins. Experiments on pH modification of the intact blue wheat kernels, to improve stability of anthocyanin pigments, indicated that slight changes can be made in which the pH could be dropped from 6.5 (original pH) to 4.4.

Three naturally-colored food products, light purplish brown, dark pink and bluish green (similar to the original grain color) were produced from blue wheat by the drum drying process. The L-, a- and b-values of the bluish green products were similar to those of blue wheat grains, while the pink product had a markedly higher a-value (redness).

We have developed naturally-colored cereal flakes from blue wheat as specialty food products

which could create a new market distinguishable from the existing one. Studying the composition and stability of anthocyanins in blue wheat will aid in the process of developing new cultivars having darker blue and more stable pigments. This will also promote manufacturing of new food products based on blue wheat.

TECHNICAL REPORT

ABSTRACT

Blue and purple wheat grains from three crop years were compared for their anthocyanin pigment composition . Blue wheat was found to have four major anthocyanins compared to five in purple wheat. Cyanidin 3-glucoside was found to be the major anthocyanin in purple wheat, while it was the second major anthocyanin in blue wheat. The major anthocyanin in blue wheat, which constituted up to 39% of total anthocynins, needs to be identified. Slight modification in pH of intact kernel could be made, e.g. from pH 6.5 to 4.4 to improve stability of anthocyanin pigments. Addition of sulfur dioxide possessed a stabilizing effect on anthocyanin pigments during heating of blue wheat whole meals or isolated anthocyanins. Three naturally-colored food products were produced based on blue wheat whole meals by drum drying process.

BACKGROUND AND OBJECTIVES

The colors of wheat grains range from white, yellow, light-red to dark-red , depending on pigment type and concentration, which are used to distinguish the major market classes of wheat. The pigmentation intensity in a particular wheat species is determined by genetic and environmental factors. Wheat pigments could be desirable or not depending on wheat species and its end-use. For instance, durum wheat with a high level of yellow pigments is considered to be a quality attribute, while red pigments in hard wheat are bleached off during the milling process. Unique genotypes of wheat with blue and purple grains have also been developed for the production of specialty wheat products. The purple pigment is located in the grain pericarp, while blue pigment is found in the aleurone layer (Zeven 1991). Early studies on red pigmentation in wheat were conducted by Nilsson-Ehle during the second decade of this century (Nilsson-Ehle 1914).

Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts which are widespread in the plant kingdom and impart attractive red and blue colours. The major anthocyanins in the pericarp of purple-grained wheat were found to be cyanidin 3-glucoside and peonidin 3-glucoside with trace amounts of the corresponding rutinosides (Dedio et al. 1972). Nowadays, there is an increasing interest in natural-colored food products. Purple wheat has been developed and utilized in New Zealand since 1980 (Bezar 1982). The grains are crushed into relatively large pieces (kibbling process) and added to the exterior of multi-grain bread. Also, blue corn is being used for the production of naturally blue-colored tortilla. Under these circumstances, developing of a dark blue-grained wheat cultivar and investigating its industrial potential will have a positive impact on cereal industry in Saskatchewan.

The major goals of the present project were to breed a blue wheat cultivar lacking red pericarp pigments, to study individual anthocyanins in blue wheat compared with purple wheat and to investigate their stability.

EXPERIMENTAL METHODS

Wheat Grains

A composite sample of spring blue wheat "Purendo 38" was obtained from selected locations in central Saskatchewan over three years (1996-98). Konini purple wheat and Katepwa, a common hard red spring wheat, were grown side by side as controls. The grains were ground to pass a 1 mm screen on a Udy Cyclone sample mill (Udy Co., Fort Collins, CO) to produce whole meal. For milling into flour, the grain samples were tempered over an 18-hr period to 14.5% moisture for blue and purple wheats and 15.5% for common wheat. The tempered grain samples were milled on a Brabender Quadrumat Jr. Flour Mill (Brabender Co., South Hackensack, NJ). The flour fractions were sifted on a 64- mesh sieve using a Ro-Tap sieve shaker (Tyler Co., Mentor, OH) for 3 min.

Analytical Tests

The pH of blue wheat and pH-modified blue wheat was measured on a 10% slurry using a pH-meter. Color of grains and food products was measured on Hunterlab Color Difference Meter, Color Quest 45/0 (Hunter Associates Laboratory Inc., Reston, VA) as L-, a- and b-values after standardization with a white color standard. Total color difference (ΔE) was calculated from means using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Where "Δ" was the difference between standard and sample readings.

Total anthocyanins was determined according to the spectrophotometric method described by Abdel-Aal et al. (1999). Individual anthocyanins in blue and purple wheats were determined by HPLC technique. Four pure anthocyanins (cyanidin 3-galactoside [Ideain], cyanidin 3-glucoside [Kuromanin], pelargonidin 3-glucoside [Callistephin], peonidin 3-glucoside) and one anthocyanidin (cyanidin) were purchased from Extrasynthese (Genay, France) and used as standards. The stock standard solutions were prepared in acidified methanol (pH 1) by weighing exactly 200-300 µg in 200 µL of acidified methanol, then 20 µL of stock solution was diluted to 500 µL with acidified methanol to prepare working standard solution. All anthocyanin standards exhibited a linear relationship with HPLC peak area in a concentration range of 0.0 to 1.0 µg (Fig. 6). The coefficients of determination (r^2) of the relationships between anthcyanin concentration and HPLC area ranged from 0.9933 to 0.9999. These calculations were based on a mixture of anthocyanin standards which was separated by HPLC using 5, 10 and 15 µL of working standard.

RESULTS

A-1 Agronomic Performance of Pigmented Wheats

Two sets of replicated field trials were conducted on pigmented wheats in 1998. Two of the lines combined the blue aleurone and purple pericarp traits, the remainder were purple pericarp lines. In the first set of trials (4 sites, 2 replications per site), 20 of the most promising lines identified in 1997 were compared to five check cultivars: Katepwa, AC Foremost, Konini, Laval 19 and Purendo 38. Konini and Laval 19 have a purple pericarp. Lines which out-yielded both purple check cultivars (Table 1) were advanced for laboratory analyses. A second set of purple breeding lines identified in unreplicated trials in 1997 were tested at two sites (2 replications per site) in 1998 (Table 2). Five of those lines out-yielded the CPS wheat check, AC Foremost. The nine most promising lines were analyzed for pigment intensity in the laboratory.

In addition to the replicated field trials a series of eight unreplicated trials were grown to assess a total of 250 pigmented breeding lines. Of those, 108 were selected for blue aleurone (BA) and white pericarp (WP) in 1997. Based on agronomic performance 39 WP/BA lines were advanced for laboratory analyses. Of the purple pericarp lines, 33 were advanced. A number of these lines will be field-tested in 1999.

In addition to the above lines, seven segregating populations combining the BA and purple or WP traits were advanced at least one generation in 1998.

A-2 Determination of Individual Anthocyanins by HPLC

Blue and purple wheat samples from the 1996, 1997 and 1998 crops were used to study their contents of individual anthocyanins by high performance liquid chromatography (HPLC). There were 5 major anthocyanins in purple wheat. Cyanidin 3-glucoside was found to be the first major anthocyanin in purple wheat constituting up to 32.7% of total anthocynins in 1996 crop and 34.7% for 1998 crop. Dedio et al. (1972) reported that the major anthocyanins in the pericarp of the purple-grained wheat line UM 606a were cyanidin 3-glucoside and peonidin 3-glucoside with trace amounts of the corresponding rutinosides as detected by paper chromatography using rhubarb and plum anthocyanins as standards. Peonidin 3-glucoside was the fifth major anthocynin in Konini purple wheat accounting for 4.8% in the 1996 crop and 4.2% in the 1998 crop. The second, third and fourth major anthocyanins were separated at retention times of 8.1, 10.0 and 12.2 min, respectively. These anthocyanins constituted 19.7, 14.9 and 5.9% of total anthocyanins in the 1996 crop and 17.2, 12.9 and 6.5% in the 1998 crop, respectively. Further study is required to identify these anthocynins. There were also trace amounts of other anthocyanins in purple wheat.

To our knowledge, there is no information available on type and concentration of anthocyanins in blue wheat. Purendo 38 blue wheat samples from three years were analyzed for their contents of anthocyanins. Four major anthocyanins were found in blue wheat. In addition, there were trace concentrations of other anthocyanins. Cyanidin 3-glucoside was found to be the second most

common anthocyanin in blue wheat constituting from 24.8 to 27.6% of total anthocynins. Peonidin 3-glucoside was found in trace amounts in blue wheat. Spiked sample with pure anthocyanin technique was used to confirm identification of these anthocyanins. The first major anthocyanin in blue wheat was eluded earlier than anthocyanin standards at a retention time of 2.3 min. This anthocyanin constituted from 37.6 to 39.3% of total anthocyanins. The other two major anthocyanins were separated at retention times of 6.1 and 8.7 min and comprised 13.0% ±0.14 and 11.1% ±0.34, respectively. The major anthocyanins in blue wheat need to be identified in a further study. Based on the results obtained, blue wheat differed in its anthocyanins composition from purple wheat. This could indicate that anthocyanin pigments differ among tissues in anthocyanin-pigmented wheats.

A-3 Modification of pH inside Intact Kernel

A composite sample of blue-grained spring wheat "Purendo 38" from the 1996 crop was used in these experiments. Our objective was to modify the pH of blue wheat kernels in order to maintain and stabilize their pigments. Buffer solutions with pH ranging from 1.0 to 10.6 were used to modify the pH inside the kernel by tempering at two levels of moisture (15 and 20%). The seeds were tempered for 16-18 hr and then dried at two different temperatures (130 °C for 1 hr and 40 °C overnight). Moisture content of the modified grains ranged from 7.2 to 9.0% in the grains dried at 130 °C and from 8.5 to 9.9% in the grains dried at 40 °C..

Despite the wide pH range used to treat the kernels, pH values of wheat slurries showed only a slight modification, ranging from 4.6 to 6.9. These slight changes might be due to indigenous buffering components and/or short tempering time. Modification at higher moisture (20%) was more effective than that at a lower tempering moisture (15%). There were slight differences in pH due to drying at high versus low temperature. There was no clear trend for the effect of pH modification on L-, a- and b-values of grain color. But, at the extreme pHs, e.g. pH 1 and pH 10.6, the grains had higher L-, a- and b-values, except for L-values at pH 10.6. This effect parallels with the nature of anthocyanin pigments which have red color in very acidic media and blue color in alkaline pH. The L-, a- and b-values of control blue wheat samples without pH modification but tempered with water to 15 and 20% moisture content and dried at 130 °C were 35.8, 1.5, 8.8 and 35.3, 1.4, 8.6, respectively versus 36.6, 1.1, 8.9 and 35.0, 1.0, 8.3 for controls dried at 40 °C.. Slight changes in total anthocyanin content were observed due to pH modification.

As we mentioned earlier, tempering for a longer time period could enhance the pH treatment effect, therefore blue wheat grains were tempered up to 4 days at pH 1 and 10.6. The pH of the control sample (tempering with water) was almost steady at 6.4-6.5. A slight decrease in the actual pH of blue wheat (4.6- 4.4) occurred when the grains tempered at 20% moisture and up to 4 days or from pH 6.5 to 5.1-4.9 when tempered at 15% moisture. On the other hand, there was no substantial change in actual pH of blue wheat samples treated with buffer pH 10.6, the pH increased from 6.5 (control) to 6.8.

Based on the results obtained, slight changes can be made in the pH of blue wheat kernels. The pH of the intact kernel could be dropped from 6.5 (original pH) to 4.4. This drop in pH would

prolong the stability of anthocyanin pigments during storage and processing of blue wheat.

A-4 Processing of Blue Wheat

Blue wheat wholemeals from the 1996 crop were treated with different food-grade chemicals and processed into flakes by drum drying. Our goal was to produce products with different natural colors. We succeeded in producing three naturally-colored food products: untreated product (light purplish brown),a dark pink product and a bluish green product (similar to the original grain color). The L-, a- and b-values of the bluish green products were similar to those of blue wheat grains, while the pink product contained markedly higher a-value (redness). The untreated product color exhibited an intermediate Hunterlab a-value.

A-5 Stability of Blue Wheat Anthocyanins

Heat stability of anthocyanins in blue wheat was studied in two different ways, as indigenous component in the whole meal and as isolated component after extraction with acidified ethanol followed by evaporation of ethanol and freeze-drying. A four-factor full-factorial experiment was used in these studies. The factors include pH (1, 3 and 5), temperature (65, 80 and 95 °C), sulfur dioxide (0, 500, 1000 and 2000 ppm in the case of whole meal and one level (3000 ppm) more in the case of isolated anthocyanin) and time (0, 1, 2, 3, 4, 5 and 6 hr). The data was statistically analyzed by General Linear Model (GLM) using Minitab Software Release 12 (Minitab, State College, PA).

The main effect of pH indicates that blue wheat anthocyanins either in whole meal or in isolated form were thermally most stable at pH 1, while their degradation was slightly lower at pH 3 compared to pH 5. As the temperature increased from 65 to 95 °C, degradation of blue wheat anthocyanins was increased as indicated by the reduction in absorbance readings. On the other hand, addition of sulfur dioxide during heating of blue wheat had a stabilizing effect on anthocyanin pigments as compared to the control sample or heating without sulfur dioxide. In the case of blue wheat whole meal, there were no significant differences between 500 ppm and 1000 ppm SO_2 in their stabilizing effect, while addition of 2000 ppm SO_2 resulted in lower stability. The reason that anthocyanin at 2000 ppm SO_2 was less stable might be attributed to irreversible bleaching effect of SO_2 at high concentration. In the case of isolated anthocyanins, the optimal SO_2 concentrations were 1000-3000 ppm. The main effect of time shows that degradation occurred at three stages having three different rates; slow rate during the first two hours of heating followed by an intermediate degradation rate during the third hour and sharp degradation during the last three hours for whole meal, while isolated anthocyanins exhibited sharp degradation followed by intermediate and slow degradations.

The interaction effects indicated that degradation of blue wheat pigments in whole meal was diminished by addition of 500 to 1000 ppm SO_2, while at a higher concentration of SO_2 (2000 ppm) the stabilizing effect was less but still much greater than the control sample (0 ppm SO_2). In the case of isolated anthocyanins, up to 3000 ppm SO_2 still had stabilizing effect on the pigments. During the first two hours of heating blue wheat whole meal at 65 °C, anthocyanins were increased because some pigments may become extractable, and after that a slight reduction in pigment content was observed

until the end of experiment time (6 hr). While in the case of isolated anthocyanins, the pigment content declined right after the first hour of heating. Heating at 80 °C resulted in a slightly higher degradation rate for anthocyanins than at 65 °C, while heating at 95 °C showed a sharp degradation. A similar trend was obtained during heating of blue wheat whole meal or isolated anthocyanin slurries at pH 1, 3 or 5 in which pigment concentration was diminished over the experiment period except for whole meal which showed an increase in pigment content during the first hour of heating at pH 1. This increase in pigment content could indicate the possibility of anthocyanin extraction continuation during the first hour of heating.

CONCLUSIONS

Blue wheat could have potential as a naturally-colored food ingredient for the cereal industry based on its anthocyanin composition. Addition of sulfur dioxide during processing of blue wheat whole meals or isolated anthocyanins is crucial for stabilization of the pigments. More research is required to identify several major anthocyanins in blue and purple wheats and to continue the breeding for developing blue wheat lacking the red pigments and containing darker blue pigments.

IMPACT ON SASKATCHEWAN AGRICULTURE

The development of blue wheat will diversify the wheat industry in Saskatchewan providing another avenue for wheat producers and processors. Blue wheat as a potential naturally-colored food ingredient for the cereal industry could create niche market for the production of specialty food products.

TECHNOLOGY TRANSFER

Abdel-Aal, E-S.M. and Hucl, P. 1999. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chem. 76: 350-354.

ACKNOWLEDGEMENTS

We would like to thank the following persons for their contributions to this project: N-Y Jai, M. Korchinsky, M. Grieman and G. Trowell.

REFERENCES

Abdel-Aal, E-S. M., Hucl, P. J. and Sosulski, F. W. 1998. Pigment composition and processing of blue-grained wheat. Soils and Crops' 98 Conference, Saskatoon, Sask., Canada, Feb. 19 & 20, 1998.

Bezar, H. J. 1982. Konini, specialty bread wheat. New Zealand Wheat Review 15: 62-63.

Dedio, W., Hill, R. D. and Evans, L. E. 1972. Anthocyanins in the pericarp and coleoptiles of purple wheat. Can. J. Plant Sci. 52: 977-980.

Nilsson-Ehle, H. 1914. Zur Kenntnis der mit der Keimungsphysiologie des Weizens in Zusammenhang stehenden inneren Faktoren. Pflanzenzuechtung 2: 153-187.

Zeven, A. C. 1991. Wheats with purple and blue grains: a review. Euphytica 56: 243-258.

ITEM "B". Deviations from original workplan - NIL

ITEM "C". Achievements - Separation and stability of blue wheat anthocyanins. Developing of naturally-colored cereal product based on blue wheat.

ITEM "D". Equipment - NIL.

ITEM "E". Personnel

M. Korchinsky - (July 1 to October 9, 1998) - 25%

M. Grieman - (October 1, 1998 to May 31, 1999) - 50%

E-S. M. Abdelaal - (April 1, 1998 to June 30, 1999) - 20%

P. Hucl - (April 1, 1998 to June 30, 1999) - 2%

Table 1. Agronomic performance in 1998 of selected lines initially tested in 1997

Cultivar	GY (kg/ha)	DSE (d)	DPM (d)	HT (cm)	TWT (kg/hL)	KWT (mg)
1 Katepwa	3548	54.8	81.6	92.9	79.3	32.6
2 AC Foremost	4550	57	88.5	72.3	80.3	40.6
3 Konini	3542	59.7	89.8	80	76.5	37
4 Laval 19	4161	59.3	87.8	75.1	74.9	35.5
5 Purendo 38	3226	58	86.6	96.1	78.3	30.8
6 PIG97003	4135	58.8	88.9	81.6	77.1	43
8 PIG97017	3791	57.5	89.3	91.4	79	34.5
10 PIG97019	4049	59	89.6	75.4	76.1	36
11 PIG97025	4198	58.7	90.1	80.6	74.8	36.6
15 PIG97036	4269	58.7	88.3	77.5	76.4	38.8
17 PIG97039	4361	59.5	89.1	81.9	77.3	40
18 PIG97040	4001	61.2	90.3	73.3	73.1	33.9
20 PIG97043	4146	57.5	89.1	76.5	75.8	41.5
21 PIG97046	4288	57.8	88.1	77.6	77.1	39.3
23 PIG97052	4154	60.5	89.6	77.8	74.6	37
25 PIG97055	4302	59	90.1	78.6	78.8	40.8
26 PIG97057	4347	59.2	89.4	77.6	77.6	37.5
# of sites	4	3	4	4	4	4

Table 2. Agronomic performance of selected new lines in 1998

Cultivar	GY (kg/ha)	DSE (d)	DPM (d)	HT (cm)	TWT (kg/hL)	KWT (mg)
1 Katepwa	3532	54.0	84.8	85.8	80.5	35.3
2 AC Foremost	4499	56.3	90.0	72.5	80.9	43.0
3 Konini	3863	58.0	90.8	84.5	76.7	35.8
4 Laval 19	4301	58.0	90.8	74.5	76.4	37.0
5 Purendo 38	3396	57.5	89.0	90.8	79.7	31.5
8 PIG98003	4689	58.8	92.3	72.8	79.2	38.5
10 PIG98005	4413	59.8	92.8	68.8	76.8	36.8
12 PIG98007	4414	59.5	91.8	74.0	76.2	38.3
18 PIG98013	4419	59.0	92.0	71.8	78.8	42.5
19 PIG98014	4450	58.5	92.3	72.5	76.5	39.8
22 PIG98017	4423	60.3	92.5	73.8	77.8	35.8
28 PIG98023	4690	57.8	91.8	71.8	81.1	43.0
29 PIG98024	4797	59.3	92.5	76.8	79.7	36.3
31 PIG98026	4523	56.0	91.5	79.0	80.2	45.8
32 PIG98027	4594	58.3	91.5	75.5	78.4	47
# of sites	2	2	2	2	2	2